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Cis-diamminedichloroplatinum(II) Augments Expression of Tumor-Associated Antigens on Human Gastric Cancer Cell Line KATO-3 and Increases Susceptibility and Binding of Tumor Cells to Various Cytotoxic Effector Cells

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Previous studies have demonstrated the immunomodulatory effects of cisplatin under certain conditions. The present study was designed to clarify whether cisplatin modulates the expression of surface antigens, especially human leukocyte antigen (HLA), on human tumor cell lines and/or augments the susceptibility and binding of tumor cells to cytotoxic effector cells. A human gastric cancer cell line, KATO-3, was employed. The expression of HLA and other tumor-associated antigens was analyzed by flow cytometry using FITC-conjugated monoclonal antibodies. The cytotoxicity of effector cells was determined by ^{51}Cr release assay. The expression of HLA class I antigen, β_2-microglobulin, leukocyte function-associated antigen-1, and AC-81 adenocarcinoma-associated antigen on KATO-3 increased after exposure to cisplatin at 10 μg/ml for 3–6 hr; augmentation of HLA class I subtypes -B2 and -B27 was particularly prominent. Furthermore, the susceptibility and binding of KATO-3 to both lymphokine-activated killer cells and KATO-3-specific cytotoxic T lymphocytes significantly increased after cisplatin treatment. Cisplatin may modulate the expression of tumor-associated antigens on some human tumor cells. Tumor regression by cisplatin administration may depend on its direct cytotoxicity as well as on its modulating effects on the expression of tumor-associated antigens, subsequently leading to the activation of the immune surveillance system against the tumor.

KEY WORDS: cisplatin, antigen modulation, chemoimmuno-modulation, human leukocyte antigen (HLA), cytotoxic T lymphocytes (CTL), lymphokine-activated killer (LAK) cell

INTRODUCTION

In general, most anticancer agents (ACAs) cause immunosuppression [1]. However, it is known that some ACAs activate the host defense mechanism under some conditions and induce specific antitumor immunity [2]. Our previous study showed that direct tumoricidal effect of ACAs may not be involved in the ultimate regression of tumor cells, which may be accomplished by augmented T-cell-mediated, antitumor immunity [3]. Accordingly, to achieve complete tumor regression, ACAs should be selected not only for their direct tumoricidal effects but also for their augmenting effects on antitumor immunity [4]. Cisplatin (CDDP) was introduced as a potent ACA

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in 1969 [5]. This agent has played an important role in the chemotherapy of various human cancers, including gastric cancer, esophageal cancer, and lung cancer. Although one of the mechanisms responsible for the antitumor activity of CDDP is thought to be direct damage to the deoxyribonucleic acid (DNA) template [6], it has been suggested that part of the antitumor activity of CDDP may arise from a host's immunological reaction to CDDP-treated cells [7]. Several studies have examined the modulatory effects of CDDP on the immunity of the host and have demonstrated that CDDP augments the antitumor immunity of the host, especially the antitumor activity of lymphocytes and macrophages [8–10]. We have studied the modulatory effects of CDDP on the antigenicity of tumor cell lines (K562 erythroleukemia and T24 urinary bladder carcinoma) and reported that the susceptibility of human tumor cells to various cytotoxic effector cells was augmented after CDDP treatment [11,12]. However, we have not clarified the mechanisms responsible for the augmentation of susceptibility of tumor cells to lysis by effector cells. In order to explain these results, we hypothesize that CDDP directly modifies and augments the expression of tumor surface antigens, resulting in the augmentation of susceptibility to cytotoxic effector cells.

The present study was designed to investigate the effects of CDDP on the expression of various surface antigens, including human leukocyte antigen (HLA) on human gastric cancer cell line, and to assess the effects of these modified surface antigens on the susceptibility and binding of human tumor cells to various cytotoxic effector cells.

MATERIALS AND METHODS

Tumor Cell Line

The KATO-3 human gastric cancer cell line was employed. KATO-3 cells are nonadherent and were maintained in complete medium: RPMI-1640 medium (Gibco, Bio-cult, Glasgow, Scotland, UK) supplemented with 25 mM HEPES (N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid], Gibco), 2 mM L-glutamine (Gibco), 1% nonessential amino acids (Gibco), 100 U/ml Penicillin (Gibco), 100 μg/ml Streptomycin (Gibco), and 10% heat-inactivated fetal calf serum (Gibco).

Treatment of Tumor Cells With Cisplatin

Commercially available cisplatin (Cis-diamminedichloroplatinum: CDDP, Randa^{R}. Lot No. 112080) was purchased from Nipponkayaku Co. (Tokyo, Japan). Recombinant human interleukin-2 (rIL-2:TGP-3, Lot No. 23206181, specific activity: 4×10^5 JRU/mg protein) was kindly supplied by Takeda Pharmaceutical Co. (Osaka, Japan). Both agents were diluted in complete medium immediately prior to the experiments. Tumor cells were treated with CDDP at the indicated concentrations in complete medium for 3 hr at 37°C. At the end of incubation, cells were washed three times in PBS, and then resuspended in either phosphate-buffered saline (PBS, Gibco) for flow cytometry or complete medium for the cytotoxicity assay, mixed lymphocyte tumor cell reaction (MLTR), and binding assay.

Preparation of Various Cytotoxic Effector Cells

Human peripheral blood was obtained from normal volunteers and from patients with gastrointestinal cancer admitted to our departments. Spleens were obtained from patients with gastric cancer who underwent gastrectomy and splenectomy as standard gastric cancer surgery. The spleen was minced with scissors in complete medium and the cell suspension was passed through a #100 mesh stainless steel screen to remove tissue fragments. Peripheral blood lymphocytes (PBL) and spleen cells (SPL) were separated by centrifugation on 100% Ficoll-Paque (Pharmacia, Uppsala, Sweden) at 400x gravity (g) for 20 min. PBL and SPL were collected from the interface and washed three times in Hanks balanced salt solution (HBSS, Gibco), and then depleted of adherent cells by incubation on plastic petri dishes in complete medium for 60 min at 37°C. Nonadherent PBL and SPL were resuspended in complete medium and were used as natural killer (NK) cell-rich lymphocytes. Flow cytometric analysis demonstrated that these nonadherent PBL or SPL usually contained 60–70% NK cells (Leu 11+) (data not shown). Lymphokine-activated killer (LAK) cells were generated by culturing nonadherent PBL or SPL in complete medium with rIL-2 at 1,000 JRU/ml for 2–3 weeks. For generating target-specific cytotoxic T lymphocytes (CTL), nonadherent PBL or SPL were cultured with target cells, which were inactivated with Mitomycin-C (MMC, Kyowa-Hakko Co., Tokyo, Japan) at 50 μg/ml in complete medium for 3 hr and then washed three times in phosphate-buffered saline (PBS, Gibco), for 3 days at a lymphocyte/tumor cell ratio of 20, 10, and 5. rIL-2 (1,000 JRU/ml) was then added into the culture system. Cells were further incubated for 3–4 weeks with periodic medium changes, and flow cytometric analysis demonstrated that these cells contained >90% CD3+ cells (data not shown).

Monoclonal Antibodies

The fluorescein isothianate (FITC)-conjugated murine monoclonal antibodies (mAbs) were obtained as follows: PHM-4 (anti-pan HLA-ABC, Lot No. QJ11A) and m3 (anti-HLA-B7 and -B27, Lot No. QI33D) were purchased from Silenus Clone (Victoria, Australia), I3 (antihuman HLA-D/DR, Lot No. I3F405) was purchased from Coulter Clone (FL), and CD54 (anti-intracellular adhesion molecule (ICAM)-1, Lot No. 10) was purchased from Immunotech (Westbrook, ME). FITC-conjugated rabbit antihuman β_2-microglobulin (β_2-MG) (Lot No. 088) and MHM24 (antihuman leukocyte function-associ-

ated antigen (LFA)-1 α-chain: CD11a, Lot No. 033) were purchased from DAKO (Glostrup, Denmark). The mouse mAb, M-58 (anti-HLA-A2, -A28) was kindly donated by Shiraimatu Shinyaku Co. (Shiga, Japan). Mouse anti-AC-81 mAb (antihuman adenocarcinoma-associated antigen) was kindly supplied by Kureha Chemical Industry Co. (Tokyo, Japan). For control staining, FITC-labeled IgG (MsIgG-FITC, Coulter Clone, Florida) was used.

Flow Cytometric Analysis

After treatment with CDDP at 10 μg/ml in complete medium for 3 hr, cells were washed three times in PBS, and 5×10^5 cells/ml were incubated with FITC-conjugated mAbs at 8°C for 30 min. After washing twice in PBS, the cells were resuspended in 1% formalaldehyde for analysis by flow cytometry (EPICS PROFILE II, Coulter Clone). Nonviable cells were gated out of the window and at least 10,000 events were accumulated using logarithmic amplification of fluorescence intensity [13].

^{3}H-Thymidine Incorporation Assay

The effects of CDDP on the growth of tumor cells were assessed by ^{3}H-thymidine (^{3}H-TdR, Amersham, Birmingshire, UK) incorporation assay [14]. Tumor cells were incubated with or without CDDP at a cell concentration of 5×10^4 cells/0.2 ml/well in a 96-well, flatbottom microtiter plate (Corning, Corning, NY). A total of 18.5 kilo Bequer (kBq) (0.5 μCi) of ^{3}H-TdR was added to each well 6 hr before harvesting the cells. After incubation at 37°C with 5% CO_2 in humidified air for the indicated periods, the cells were harvested onto glass fiber filters (Labo Science, Tokyo, Japan) by a semiautomatic cell harvester (Titertek, Flow Laboratories, Rickmansworth, Herts, UK). Before harvesting the cells, the medium in the wells was carefully aspirated with a pipet, and 100 μl of trypsin-ethylenediamine-tetraacetate (EDTA) solution (0.25% trypsin, 1 mM EDTA, Gibco) was added to the wells to remove adherent cells from the plastic. The plate was then shaken on a plate mixer (Iwaki, Funabashi, Japan) at 37°C for 5–10 min, and the harvesting procedure was performed. After drying at room temperature, the glass fiber filters were placed in scintillation vials (Packard, Meriden, CT) with 3 ml of liquid scintillation fluid (PCS, Amersham), and the radioactivity was measured as cpm (counts per min) using a liquid scintillation counter (Aloka LSC-1000, Tokyo, Japan). All experiments were set up in triplicate.

Mixed Lymphocyte Tumor Cell Reaction (MLTR)

Effector cells were adjusted to 1×10^6/ml in complete medium, and 0.1 ml of cell suspension was pipetted into a 96-well, flatbottom microtiter plate at 1×10^5/well. As stimulator cells, X-irradiated (1,500 rads) tumor cells (5×10^3/0.1 ml) with or without CDDP-treatment were added into each well at an effector/target (E/T) ratio = 20. These cells were cultured at 37°C with 5% CO_2 in humidified air for 3 days. ^{3}H-TdR was added at 18.5 kBq/25 μl PBS/well for the last 18 hr of culture and the cells were harvested onto the glass fiber filters with a semiautomatic cell harvester. After drying at room temperature, the glass fiber filters were placed into scintillation vials containing 3 ml of scintillation fluid, and their radioactivity was measured in a liquid scintillation counter. All tests were set up in triplicate and the mean values were used in calculations.

Cytotoxicity Assay

Target cells were labeled with ^{51}Cr by incubating cells with $Na_2\,{}^{51}CrO_4$ (Amersham) at 3.7 M(mega)Bq (100 μCi)/10^6/ml for 3 hr at 37°C in a humidified 5% CO_2 atmosphere. After incubation, the cells were washed three times in HBSS and resuspended at a concentration of 5×10^4/ml in complete medium. These labeled target cells were treated with 10 μg/ml CDDP for 3 hr and washed three times in HBSS; 100 μl of target cell suspension (5×10^3 cells) and 100 μl of effector cell suspension were added at an E/T ratio of 20 in a 96-well, round bottom microtiter plate (Corning). Each plate was incubated for 12 hr at 37°C in a humidified 5% CO_2 atmosphere. After incubation, the supernatant was collected with Titertek-Supernatant Collection System (Titertek), and the ^{51}Cr radioactivity was measured in an autogamma scintillation counter (Minaxi, Packard, Tokyo, Japan). Spontaneous ^{51}Cr release was determined in the wells containing 100 μl of target cell suspension and 100 μl of medium, and maximum release was determined by the addition of 100 μl of 2% Triton X-100. The % specific cytotoxicity for each assay was determined according to the following formula:

% specific cytotoxicity = [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100.

All experiments were set up in triplicate and the mean values were used in calculations.

Binding of Effector Cells to Tumor Cells

Equal numbers (2×10^5) of effector cells and tumor cells were mixed in 0.2 ml of medium in 15 ml round bottom plastic tubes (Corning), and incubated for 10 min at 37°C in a humidified 5% CO_2 atmosphere. The tube was then centrifuged at 100xg for 5 min at 8°C, and then 0.8 ml of complete medium was carefully added. The pellet was carefully pipetted twice with a Pipetboy (TEC NO MARA, Zurich, Swiss), and 1 ml of cell suspension was dropped on to the ice-cold monolayer of agar on a 12-well plate dish (Corning). The plates were centrifuged at 100 xg for 5 min at 8°C. The percentage of effector cells binding to target cells was determined by counting at least 200 effector cells using a phase microscope [12].

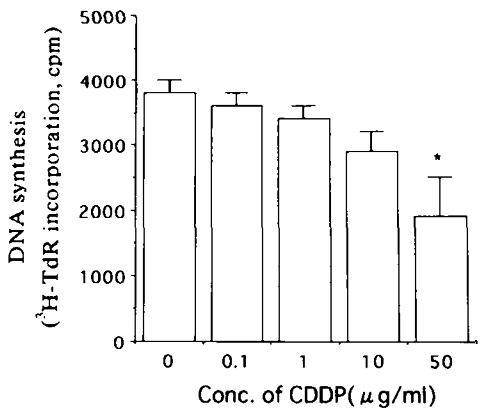

Fig. 1. Effect of cisplatin on DNA synthesis in KATO-3 cells. The in vitro effect of cisplatin on DNA synthesis in KATO-3 cells was assessed by ^{3}H-thymidine incorporation assay. Cells were incubated with cisplatin for 24 hr, pulsed with ^{3}H-thymidine during the last 6 hr of culture, and harvested. Incorporated ^{3}H-thymidine was measured as described in Materials and Methods. Bars indicate the standard deviation. *, $P < 0.05$ vs. control (medium alone)

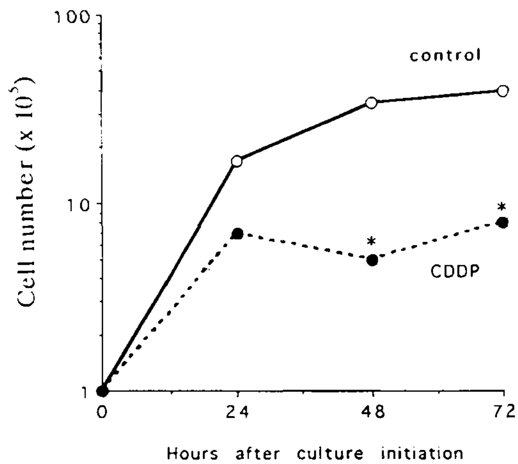

Fig. 2. Effect of cisplatin on growth of KATO-3 cells. Cells (1×10^5) were incubated with cisplatin at 10 μg/ml in 10 ml of complete medium at 37°C and viable cells were counted by the trypan blue dye exclusion test at the indicated time after culture initiation. —○—, control (medium treatment); · · · ● · · ·, cisplatin treatment. *, $P < 0.01$ vs. control.

Statistical Analysis

All cytotoxic assays were set up in triplicate, and the mean ± standard deviation was used. Statistical significance was determined by Student's *t*-test.

RESULTS

Effects of CDDP on DNA Synthesis and Growth of KATO-3 Cells

The effect of CDDP on DNA synthesis in KATO-3 cells was assessed by ^{3}H-TdR incorporation assay, and the results are shown in Figure 1. CDDP at 0.1–10 μ/ml showed no significant inhibitory effect on DNA synthesis in KATO-3 cells during 24 hr of incubation; however, CDDP at 50 μg/ml significantly inhibited DNA synthesis.

The effect of CDDP on the growth of KATO-3 cells was assessed by trypan blue dye exclusion assay, and the results are shown in Figure 2. Incubating KATO-3 cells with CDDP at 10 μg/ml for >2 days resulted in a significant inhibition of growth. The viability of all cultured cells, which was assessed by the trypan blue dye exclusion test, was usually >95%. There was no difference in viability between the control and the CDDP-treated KATO-3 cells.

Tumor cells were treated with CDDP at 10 μg/ml for 3 hr in subsequent experiments in order to exclude the direct cytotoxic effect of CDDP on tumor cells.

Expression of HLA Class I and II Antigens on KATO-3 Cells After CDDP Treatment

The changes in the expression of HLA class I and II antigens on KATO-3 cells after CDDP treatment are summarized in Figure 3. HLA class I and class II antigens were originally expressed on the control KATO-3 (KATO-3 without CDDP treatment). After CDDP treatment the expression of HLA class I antigen on KATO-3, as repre-

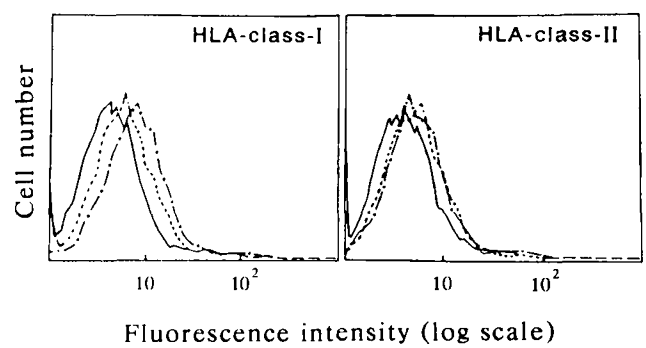

Fig. 3. Flow cytometric analysis of the expression of HLA class I and II antigens on KATO-3 cells. Cells were treated with cisplatin at 10 μg/ml for 3 hr and then washed three times with phosphated-buffered saline. The cells were recultured with complete medium for 6 or 12 hr and then reacted with FITC-labeled monoclonal antibody PHM-4 and I3, which are specific for HLA-A,B,C and HLA-D/DR, respectively. Flow cytometry was then performed as described in Materials and Methods. Each determination was carried out a minimum of three times. ——, control (medium treatment); – – –, cisplatin treatment (6 hr after); – · – ·, cisplatin treatment (12 hr after).

sented by the mean channel number, was augmented twofold compared to the control KATO-3 between 6 and 12 hr after CDDP treatment. However, the expression of HLA class II antigen was not enhanced.

Expression of Polymorphic HLA Class I Antigen, Cell Binding-Associated Molecules, and AC-81 Adenocarcinoma-Associated Antigen on CDDP-Treated KATO-3 Cells

HLA-A2, -A28, and -B2, -B27 subtypes of class I antigens on tumor cells were assessed. As shown in Figure 4, the expression of all subtypes of HLA class I antigen on KATO-3 was enhanced by CDDP, to almost the same extent. In particular, B27 and AC-81 expression was sig-

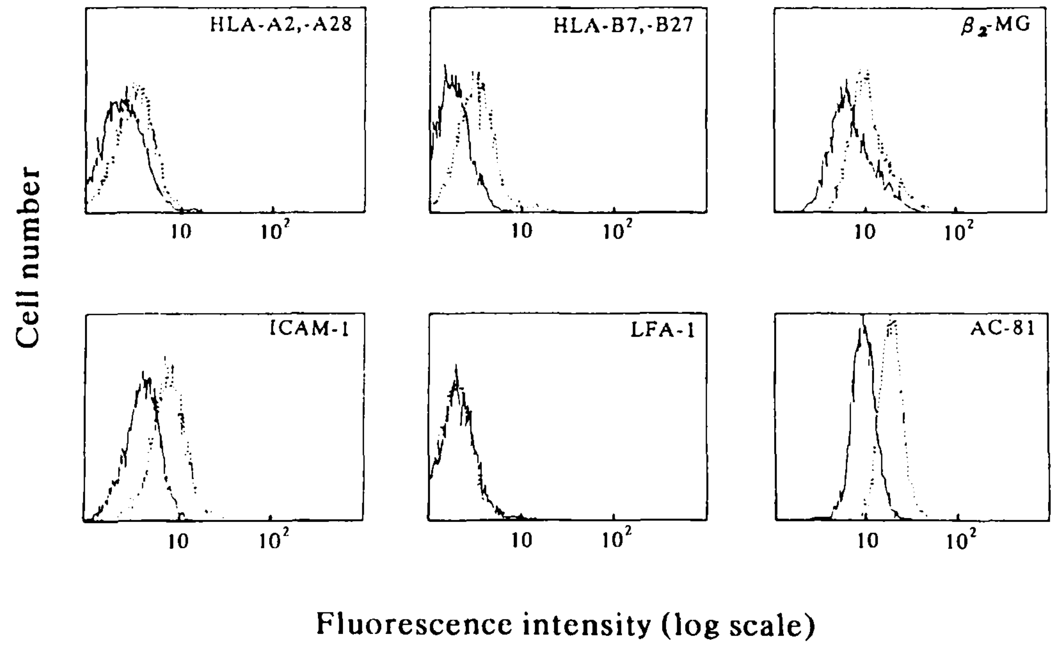

Fig. 4. Constitutive expression of subtypes of HLA class-I antigen, β2-microglobulin, ICAM-1, LFA-1, and AC-81 on KATO-3 cells. Cells were treated with cisplatin at 10 μg/ml for 3 hr, and washed three times with phosphate-buffered saline. After culturing with medium for 6 hr, the cells were treated with FITC-labeled mAbs and then analyzed by flow cytometry as described in Materials and Methods. Each determination was carried out a minimum of three times. ——, control (medium treatment); · · · ·, cisplatin treatment.

nificantly increased. Furthermore, CDDP augmented the expression of β_2-MG on KATO-3. The expression of the cell-adhesion molecule, ICAM-1, on KATO-3 was enhanced by CDDP treatment, and adenocarcinoma-associated antigen (AC-81) expression on KATO-3 was also enhanced by CDDP.

Stimulation of Effector Cells by CDDP-Treated KATO-3 Cells

Nonadherent PBL or SPL (NK rich fraction), LAK cells and CTLs were stimulated by X-irradiated, CDDP-treated KATO-3 cells for various lengths of time using MLTR assay. Figure 5 details the duration of stimulus required to generate a response. The stimulation of LAK cells and KATO-3-specific CTL by CDDP-treated KATO-3 for 3–24 hr resulted in a significant increase in ^{3}H-TdR incorporation as compared to the stimulation by control KATO-3 (irradiated KATO-3 without CDDP-treatment). The increased stimulation was dependent on the duration of stimulus. NK cells were not significantly stimulated by control or CDDP-treated KATO-3 (data not shown).

Target Susceptibility to Lysis by Effector Cells After Exposure to CDDP Treatment

The viability of KATO-3 after incubation with CDDP at 10 μg/ml for 3 hr was >95% and was comparable to CDDP-nontreated tumor cells. Incubation with CDDP at 10 μg/ml for 24 hr had no effect on viability and recovery. However, culture with CDDP for >2 days resulted in a significant decrease in cell number (Fig. 2). As shown in Fig. 6, KATO-3 incubated with CDDP for 3 hr demonstrated an increased sensitivity to lysis by LAK cells and KATO-3-specific CTL, but their susceptibility to lysis by nonadherent PBL or SPL was not augmented.

Furthermore, with anti-HLA class I mAb was added to the cytotoxicity assay system, the lysis of CDDP-treated KATO-3 by LAK cells or CTLs significantly decreased (Fig. 7).

Binding of CDDP-Treated KATO-3 Cells With Effector Cells

After treating KATO-3 with CDDP, the binding of LAK cells or KATO-3-specific CTL with CDDP-treated KATO-3 was significantly increased in comparison with their binding with control KATO-3 (CDDP nontreatment). However, the binding of nonadherent PBL or SPL to KATO-3 cells was not augmented after CDDP-treatment (Fig. 8).

DISCUSSION

It has been proposed that the anticancer potential of CDDP includes its immunomodulatory effects on the host's immune system, which enhance the response to

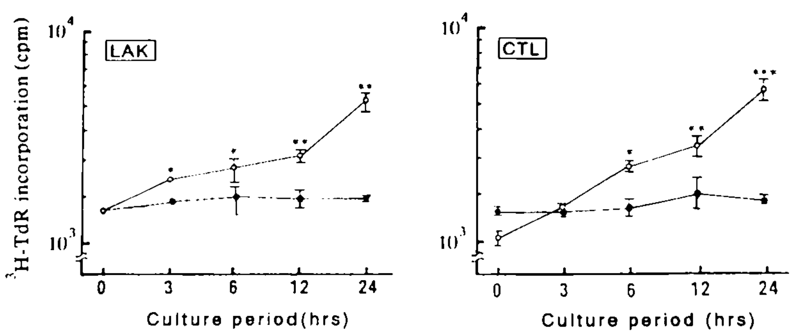

Fig. 5. Kinetics of mixed lymphocyte tumor cell reaction (MLTR). Lymphokine-activated killer (LAK) cells and KATO-3-specific cytotoxic T-lymphocytes (CTL) were cultured with cisplatin treated irradiated KATO-3 cells or with cisplatin nontreated irradiated KATO-3 cells (control). The response of the effector cells was assessed by measuring ^{3}H-thymidine incorporation into the effector cells after culturing with KATO-3 cells as described in Materials and Methods. The X-axis indicates the culture duration of MLTR, and bars indicate the S.D.. —○—, CDDP treated KATO-3; —●—, control KATO-3. *,**,***$P < 0.05$, 0.01, and 0.005 vs. control.

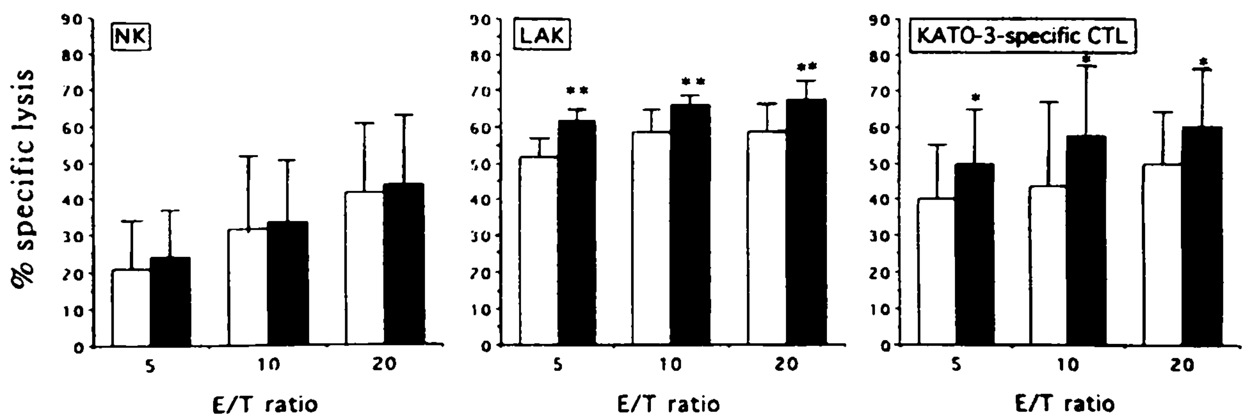

Fig. 6. Effects of CDDP on the susceptibility of KATO-3 cells to lysis by various effector cells. Cells were incubated with medium alone (control) or with cisplatin at 10 μg/ml for 3 hr at 37°C, washed, and then subsequently used as targets for each of the various effector cells at an effector/target ratio of 20, 10, and 5 in a 12-hr ^{51}Cr-release assay, as described in Materials and Methods. Results are expressed as % specific lysis. Bars indicate the standard deviation. Natural killer (NK) cell-rich fraction (fresh peripheral blood lymphocytes or splenic lymphocytes), n = 17; lymphokine-activated killer (LAK) cells, n = 7; KATO-3-specific cytotoxic T lymphocytes (CTL), n = 7. □, control (medium treatment); ■, cisplatin treatment. *, $P < 0.1$; **, $P < 0.05$ vs. control.

autochthonous tumors [15]. Our previous studies also have suggested that the activation of the host defense mechanism by CDDP may also contribute to platinum-mediated tumor cytoreduction [11]. In addition to its effect on immune activity, CDDP has also been shown to directly stimulate spontaneous human monocyte-mediated cytotoxicity [16]. It was also reported that incubation of YAC-1 targets with CDDP for 1 or 18 hr significantly enhanced their lysis by normal spleen cells [17]. It was hypothesized that CDDP may alter membrane components of tumor cell, leading to increased recognition of targets by effector cells, enhanced target cell binding, and subsequent increased lysis. The present results also support these hypotheses.

Human MHC class I antigens are composed of polymorphic HLA-A, -B, -C, and H chains are associated with an invariant L-chain and β_2-MG [18]. In particular, polymorphic residues of B-27 play a crucial role in peptide binding and in the stability of peptide-MHC class I complexes [19]. HLA class I antigen exerts its function in association with β_2-MG to direct CTL to attack virally infected or neoplastic cells [17]. However, MHC class II molecules act as restriction elements for helper T cells [20]. Recently, it was postulated that expression of HLA B-7 in tumor cells caused the recognition of foreign antigen by the immune system [21]. It was suggested that the absence of MHC class I antigen expression may correlate with greater tumor aggressiveness in several tumors [22].

In this study we investigated the effects of CDDP treatment on HLA surface antigens on KATO-3 human gastric cancer cells and on the susceptibility of the cells to lysis by various effector cells. Gastric cancer was chosen to study since it is a malignancy that is very sensitive to

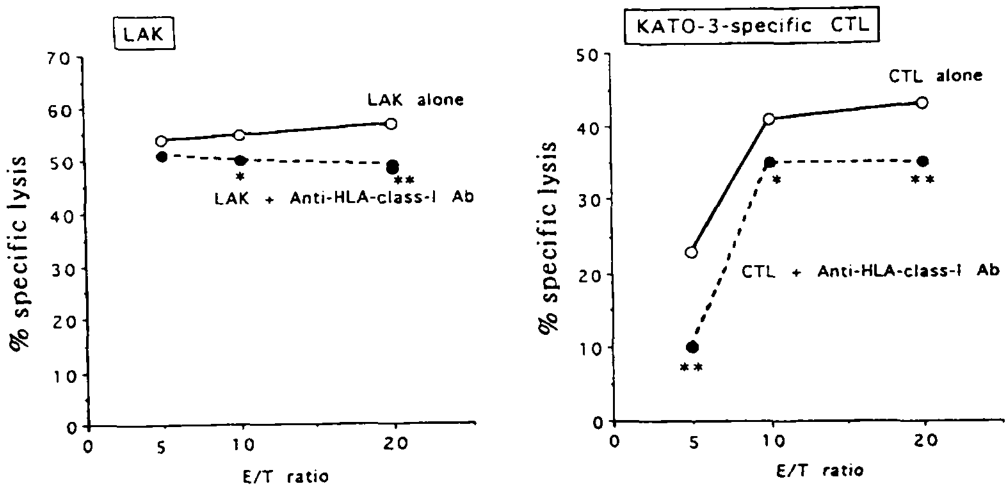

Fig. 7. Effect of anti-HLA class-I antibody on the susceptibility of KATO-3 cells to lysis by lymphokine-activated killer (LAK) cells and cytotoxic T-lymphocyte (CTL). Anti-HLA class I monoclonal antibody was added to the cytotoxicity assay system of ^{51}Cr-release at a 50:1 dilution, and the % specific lysis was assessed as described in Materials and Methods. —○—, Target + effector cell. ···●···, Target cell + effector cell + anti-HLA class-I monoclonal antibody. *, $P < 0.05$; **, $P < 0.01$.

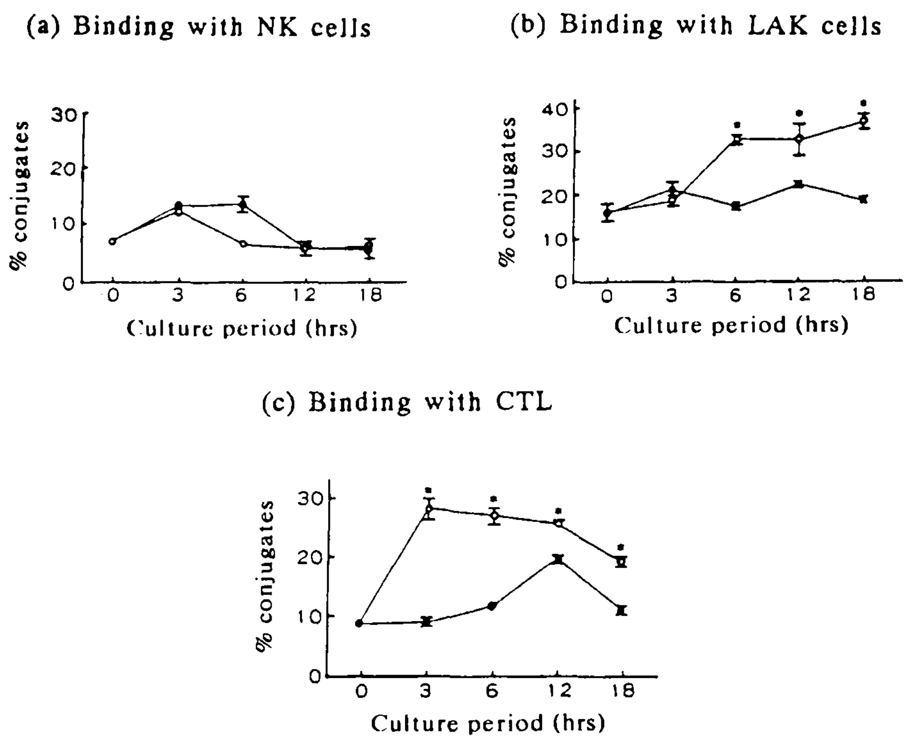

Fig. 8. Effects of cisplatin on binding of KATO-3 cells to effector cells. Cells were incubated with medium alone (control) or with cisplatin at 10 μg/ml for 3 hr at 37°C, washed, and then cocultured with various effector cells. The number of effector cell/KATO-3 cell conjugates was assessed using a single cell binding assay, as described in Materials and Methods. Bars indicate the standard deviation. The X-axis indicates the duration of coculture with effector cells and KATO-3 cells. —●—, control; —○—, cisplatin treatment. *, $P < 0.01$ vs. control.

CDDP in clinical chemotherapy. HLA class I antigen expression on KATO-3 and the susceptibility of the cells to LAK cells and KATO-3-specific CTL were simultaneously augmented after CDDP treatment, as compared with the controls (Figs. 3, 6). It is not clear whether this increased susceptibility of CDDP-treated KATO-3 cells

to LAK cells and KATO-3-specific CTL is always dependent on augmented HLA class I antigen expression on the cells. However, the present result demonstrates that augmented HLA class I antigen expression on tumor cells resulting from CDDP exposure makes tumor cells easier to recognize for effector cells and facilitates the binding between effector cells and tumor cells, leading to increased lysis by effector cells. In fact, modified membrane components derived from irradiated CDDP-treated KATO-3 stimulated LAK cells and KATO-3-specific CTL (Fig. 5). HLA class I antigens specifically regulate the ability of CTL to recognize antigens [17]. Accordingly, our result showing that the increased susceptibility of KATO-3 to KATO-3-specific CTL is associated with augmented expression of HLA class I antigen is reasonable (Figs. 6, 7). However, it is thought that LAK cells exhibit MHC-unrestricted cytotoxicity [23]. According to this immunokinetic characterization of LAK cells, it is not reasonable that the augmented expression of HLA class I antigen on CDDP-treated KATO-3 caused an enhancement in susceptibility to LAK cells. However, several investigators reported that a short term (1–2 weeks) culture of PBL with IL-2 induces NK cell dominant LAK (NK-LAK) and a long-term (>3 weeks) culture induces T-cell dominant LAK (T-LAK), which may include CTLs [5]. In the present study we generate a LAK cells by culturing cells for 2–3 weeks. Accordingly, it is reasonable that the susceptibility of CDDP-treated KATO-3 to lysis by long-term cultured LAK cells would be augmented. The present study also demonstrated that the augmented expression of HLA class I antigen by CDDP had no effect on the susceptibility of KATO-3 to NK cells. It is also reported that the recognition of target cells by NK cells are not restricted to HLA class I antigens and that there appeared to be an inverse correlation between sensitivity to NK cells and HLA class I expression [24]. However, other recent reports found that HLA class I antigen expression had no influence on NK cell-mediated cytolysis [25,26]. These results are also compatible with those reported here.

According to our present understanding, β_2-MG is required for posttranslational processing and the transport of all HLA-A, -B, -C gene products to the cell surface [27]. The selective elimination of β_2-MG expression represents a unique mechanism leading to a complete loss of class I antigen expression in vivo [28]. Cell surface expression of class I antigen requires the intercellular assembly of H- and L-chains and β_2-MG [18]. In this study the expression of β_2-MG as well as HLA class I antigen was augmented by CDDP-treatment in KATO-3. This suggests that a simultaneous increase in both HLA class I molecule expression and β_2-MG molecule expression may be essential for the recognition of tumor cells by effector cells.

The present study using anti-HLA class I mAb in a cytotoxicity assay (Fig. 7) also demonstrated that augmented HLA class I antigen expression is partially responsible for augmented lysis by LAK cells or CTLs and that other surface molecules may be associated with target cell recognition by effector cells. The structure involved in nonspecific target cell recognition by T cells remains largely unknown. However, structures involved in cell adhesion have been identified on the surface of leukocytes. Of these surface antigens, LFA-1 is the major leukocyte integrin on lymphocytes [29] and one of the ligands for LFA-1 is ICAM-1. The interaction between these adhesion molecules is important for binding of tumor cells and effector cells to occur in the event of effector cell-mediated tumor regression. Anti-LFA-1 and anti-ICAM-1 mAbs block CTL, NK, or LAK-mediated target cell lysis [16]. After viral infection, astrocytes become more susceptible to alloantigen-specific cytolysis by T lymphocytes, and CTL activity was substantially reduced in the presence of mAbs specific for MHC class I, ICAM-1, and LFA-1, but not for MHC class II [30]. In the present study, LFA-1 on KATO-3 cells was not augmented after CDDP treatment; however LFA-1 is mainly expressed on lymphocytes. Since the expression of ICAM-1, which is a ligand of LFA-1, on KATO-3 cells was augmented and binding of tumor cells and effector cells also increased after CDDP treatment, it is suggested that augmented expression of ICAM-1 on KATO-3 cells after exposure to CDDP also may play an important role in binding and recognition between KATO-3 cells and T lymphocytes (Figs. 4, 8). Furthermore, the expression of adenocarcinoma associated antigen, AC-81 was also augmented after CDDP treatment, which also may be responsible for augmented tumor recognition by cytotoxic effector cells after CDDP treatment.

After CDDP treatment, the expression of HLA class I and other surface antigens was rapidly augmented after just 3 hr of exposure (usually <6 hr). The exposure was too short for de novo protein synthesis, and this may suggest an intracellular pool of surface antigens. We hypothesize the following possible mechanisms for this phenomenon: (1) intracellularly pooled cell-surface antigens are induced to travel to the cell surface after cell membrane has been damaged by CDDP; (2) surface proteins or glycolipids that cover surface antigens are removed by CDDP; (3) HLA class I antigen and β_2-MG, both of which are already weakly expressed on the tumor cell surface, are cross-linked by CDDP, subsequently leading to an augmentation of antigenicity.

It has been reported that CDDP enhances the release of various cytokines, especially TNF and IL-1, from effector cells [31,32]. This also suggests that CDDP has a variety of mechanisms responsible for activating host defense systems and one mechanism may be mediated via stimulation to secrete different kinds of cytokines.

In summary, the antigen-modulating effect of CDDP

may be an important mechanism responsible for the antitumor activity of CDDP.

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